Epithelial-to-mesenchymal transition in a fistula-associated anal adenocarcinoma in a patient with long-standing Crohn's disease

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Abstract: Anal adenocarcinomas arising from perianal fistulae represent a rare complication in Crohn's disease (CD) patients. We have previously demonstrated the involvement of an epithelial-to-mesenchymal transition (EMT) in the pathogenesis of CD-associated fistulae. Although EMT has also been implicated in the development of colorectal and anal carcinoma, the molecular link from fistula to carcinoma is unclear. We present a case of a 48-year-old White woman who developed a mucinous anal adenocarcinoma originating from a perianal, CD-associated fistula 24 years after being diagnosed with CD. To characterize the expression of EMT-associated molecules in fistula and carcinoma tissue, immunohistochemical analysis for Snail1, Slug, -catenin and E-cadherin was performed. A mucinous anal adenocarcinoma developed on a perianal fistula in a patient with long-standing CD. After neoadjuvant radiochemotherapy, the fistula-associated tumour was resected and the patient is presently in remission. Using immunohistochemical analysis, we detected a remarkable staining of the Slug transcription factor in transitional cells lining the fistula tract. This observation is unique to this 'carcinoma'-fistula: we had previously shown Slug expression in cells surrounding the fistula tract but not in transitional cells. Expression of Snail1, -catenin and E-cadherin in this case was comparable with our previous findings. We describe a rare case of a CD fistula-associated adenocarcinoma within an area of squamous epithelium of the perianal area and an unusual expression pattern of EMT markers in this fistula. This case seems to underline the relevance of our previous findings demonstrating that EMT plays an important role for fistula pathogenesis and likely carcinogenesis in CD patients.

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Anal adenocarcinomas arising from perianal fistulae represent a rare complication in Crohn's disease (CD) patients. We have previously demonstrated the involvement of an epithelial-to-mesenchymal transition (EMT) in the pathogenesis of CD-associated fistulae. Although EMT has also been implicated in the development of colorectal and anal carcinoma, the molecular link from fistula to carcinoma is unclear. We present a case of a 48-year-old White woman who developed a mucinous anal adenocarcinoma originating from a perianal, CD-associated fistula 24 years after being diagnosed with CD. To characterize the expression of EMT-associated molecules in fistula and carcinoma tissue, immunohistochemical analysis for Snail1, Slug, β-catenin and E-cadherin was performed. A mucinous anal adenocarcinoma developed on a perianal fistula in a patient with long-standing CD. After neoadjuvant radiochemotherapy, the fistula-associated tumour was resected and the patient is presently in remission. Using immunohistochemical analysis, we detected a remarkable staining of the Slug transcription factor in transitional cells lining the fistula tract. This observation is unique to this 'carcinoma'-fistula: we had previously shown Slug expression in cells surrounding the fistula tract but not in transitional cells. Expression of Snail1, β-catenin and E-cadherin in this case was comparable with our previous findings. We describe a rare case of a CD fistula-associated adenocarcinoma within an area of squamous epithelium of the perianal area and an unusual expression pattern of EMT markers in this fistula. This case seems to underline the relevance of our previous findings demonstrating that EMT plays an important role for fistula pathogenesis and likely carcinogenesis in CD patients. *Eur J Gastroenterol Hepatol* 26:114–118 © 2013 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Introduction

Crohn's disease (CD), a subtype of inflammatory bowel disease, is characterized by a chronic and transmural inflammation of the gastrointestinal (GI) tract. One of its most important complications is the development of CD-associated fistulae that can occur anywhere along the GI tract. According to population-based cohorts, the cumulative incidence of fistulas in CD patients is 17–50%. The most frequent fistulas are perianal (54%), enteroenteric (24%) and rectovaginal (9%) [1]. However, fistulae represent a recurring complication in about one-third of affected patients, making them difficult to treat. Although new and more effective medications, such as antitumour necrosis factor (anti-TNF) antibodies, have been developed for inflammatory bowel disease and CD in particular, the outcome of medical fistula therapy is still poor and surgery is often needed [2,3].

A rare complication of CD is represented by carcinomas arising from perianal fistulas, in particular anal adenocarcinoma. Such adenocarcinomas develop in an area of squamous epithelia such as the perineum. As CD fistula tracts are either covered by squamous cells or flat cells – attributed as 'mesenchymal cells' until we identified them as epithelial-to-mesenchymal transition (EMT)-transformed intestinal epithelial cells (IECs) – the origin of the adenocarcinoma cells on the skin surface was so far unclear. To date, less than 70 cases of CD fistula-associated adenocarcinomas have been described in the literature [4]. The presence of such CD-associated anal adenocarcinomas is clearly associated with long-standing perianal fistulizing disease [5]. The outcome for patients with fistula-associated anal adenocarcinoma is generally poor, especially in patients with node-positive carcinoma, featuring an overall survival rate of 54% [4]. Here, we report the case of a 48-year-old White woman suffering from severe fistulizing CD who developed a mucinous adenocarcinoma of the anus originating from a CD-associated fistula tract 24 years after being diagnosed with CD.

Case report

In December 2009, a 45-year-old female patient was referred to our outpatient clinic as she had active, severe, fistulising CD with persistent massive abdominal pain

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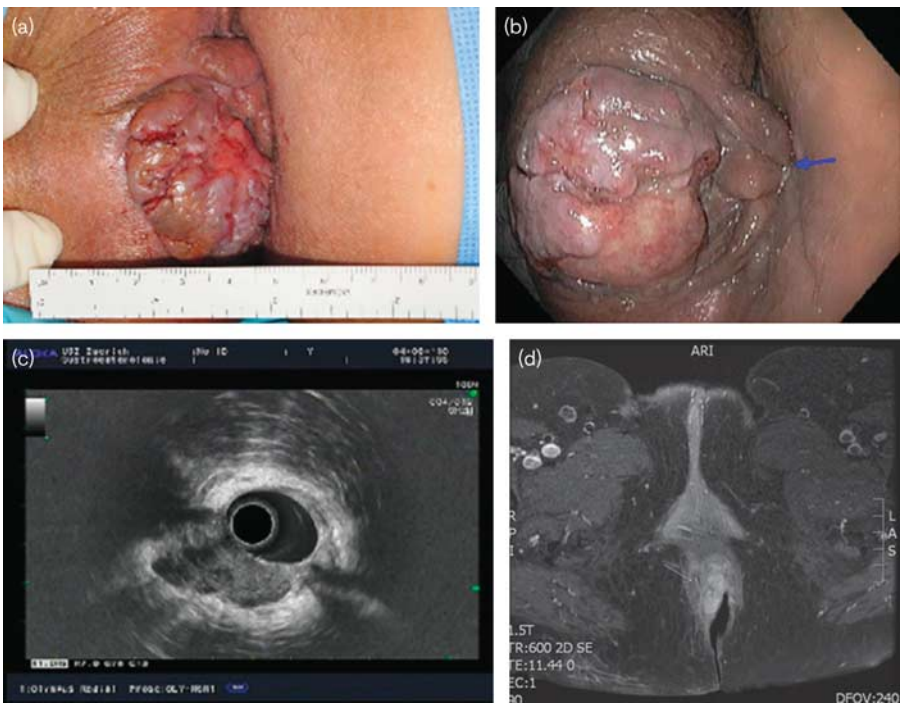
and a perianal fistula despite a 10-month treatment with certolizumab pegol.

The patient had first been diagnosed with CD in 1985 at the age of 20. In 1986, an ileocoecal resection was performed as stenosis of the ileum was observed. In 1993, a perianal abscess accompanied by a rectovaginal fistula occurred, and in 1998 it was finally surgically removed owing to the ongoing complications. Because a stenosis was present in the area of the ileoascendostomy, a follow-up resection had to be performed. From 2002 to 2006, the patient suffered from recurrent episodes of severe pain in the epigastric region, along with nausea, vomitus and diarrhoea. Finally, in March 2006, a capsule endoscopy was performed, which revealed severe inflammation and stenosis in the small intestine. Consequently, the immunosuppressive medication was switched from 6-mercaptopurin to methotrexate (MTX). As the patient began suffering from even more abdominal pain after the injections, MTX was stopped in November 2006, and therapy with infliximab was initiated in January 2007. In the following months, the perianal fistulas partially declined; however, the severe abdominal pain was persistent, and in October 2007 infliximab was finally stopped. In autumn 2008, an acute flare of CD was successfully treated with budesonide administered orally. Nevertheless, as the abdominal pain became even worse, in February 2009, a second anti-TNF therapy was started with certolizumab pegol. However, the abdominal pain and perianal fistulas remained unaltered.

On initial presentation at our outpatient clinic, we detected an acute CD flare in the region of the terminal ileum with a bowel wall thickening of up to 8 mm determined ultrasonographically and decided to switch therapy to adalimumab as a third anti-TNF medication. In the following months, the abdominal pain clearly improved, but diarrhoea was still persistent to some extent; additional therapy with budesonide was started finally, which caused normalization of stool consistency within 6 months after start of adalimumab. As the complaints from the perianal fistulae still persisted, antibiotic treatment with ciprofloxacin and metronidazole was started.

In February 2010, a small perianal tumour in the fistula region was seen and biopsied. Histopathological assessment revealed a florid erosion and ulceration next to a chronically granulating inflammation with embedded excavations that were lined with cubic and cylindrical epithelium. No malignant cells were detected. As the swelling progressed, in July 2010, an MRI of the pelvis was performed; a complex fistula system with a perianal tumour on the right side with a size of $3.8 \times 2.3 \times 3.5$ cm

Fig. 1



Fistula-associated perianal adenocarcinoma. The tumour has been shown (a) macroscopically, (b) by means of colonoscopy (blue arrow points onto the fistula opening), (c) by endosonography and (d) by MRI scan.

was thus detected. From the tumour, a fistula was growing towards the anal part of the rectum (Fig. 1). An additional biopsy was performed. Histopathological assessment revealed that the tumour was a poorly differentiated mucinous adenocarcinoma of the anus arising from the fistula tract. On the PET-CT scan, no lymph node or solid organ metastases were detected; endosonography revealed a connection of the tumour to a transsphincteric fistula and tumour infiltration into the subcutis. Adalimumab was continued. Neoadjuvant radiochemotherapy was performed in August/September 2010. Radiotherapy of the primary tumour consisted of 25 sessions applying 1.8 Gy each, accompanied by concomitant chemotherapy with 5-fluoruracil, which was poorly tolerated. In the following weeks, an acute Crohn's flare occurred, which could be confirmed by MRI, endoscopy and histopathological assessment. The tumour lesion was smaller in size. In November 2010, amputation of the rectum was performed. The tumour stage was finally determined to be ypT2, ypN0 (0/14), cM0, R0, and the tumour was proved histopathologically as a mucinous adenocarcinoma of the perineum. Adalimumab was preoperatively stopped and suspended because of an increased risk of infectious complications during surgery and the potential effect on the development of malignancies. Budesonide was continued. In January 2011, an acute CD flare was sonographically diagnosed, treatment with MTX was reinitiated; this time it was well tolerated, and the health condition improved. However, the patient again presented with abdominal pain and radiological signs of a subileus due to a structuring lesion observed at ileocoecostomy, which was finally resected in November 2011. Since then, the patient is fairly stable and in good condition; she is under therapy with intermittent budesonide courses. Anti-TNF medication has not been resumed. To date, no relapse of the anal carcinoma was observed.

Discussion

In our case, a fistula-associated mucinous adenocarcinoma of the perineum arose in a patient suffering from CD for 25 years. Although mainly patients with ulcerative colitis are regarded to be at increased risk for developing colorectal cancer, the relative risk for CD patients to develop small bowel cancer is 28.4-fold and that of colorectal cancer is 2.4-fold higher compared with the normal population [6]. There is a significant association between the site of inflammation within the GI tract and the risk of cancer in the respective part as well as between the duration of CD and the cancer risk [6,7].

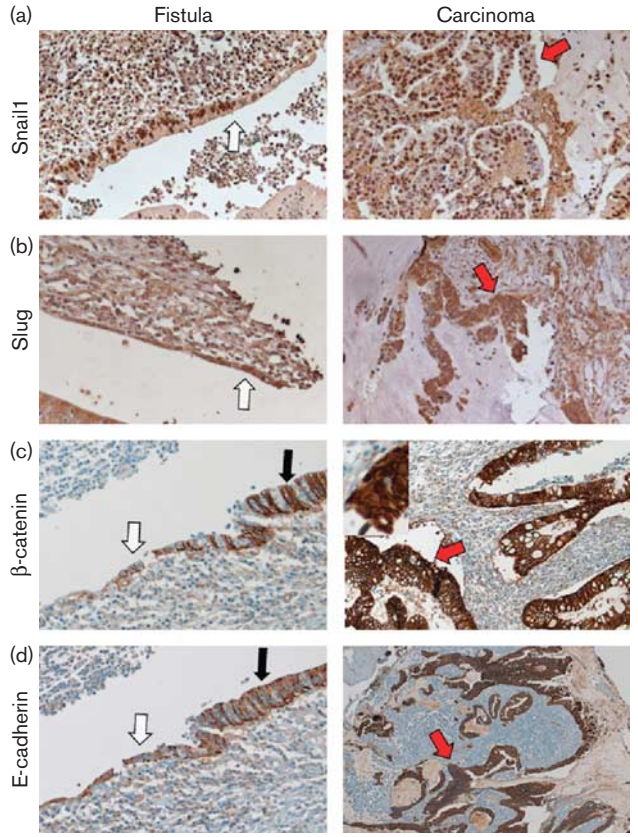
Chronically ongoing fistulizing inflammation carries an elevated risk of malignant transformation, regardless of the anatomical location [8]. The average patient developing a CD fistula-associated carcinoma would have been suffering from CD for 24 years, from perianal fistulas for 14 years and from perianal disease for at least 10 years [4]. The majority of patients seem to be female (57%; mean age: 53 years). Our 45-year-old patient might thus be a 'typical' patient suffering from CD for 24 years with a history of perianal, fistulizing disease of more than 15 years. Her perianal fistulas were extensively treated but were recurrent after surgery, not responding to any medical treatment, and featured throughout signs of inflammation such as abscesses and pain, which are also the most common symptoms of the patients reported in the literature [4].

In our case, an initial biopsy of the suspicious region was performed; however, no signs of malignancy were detected, and no malignancy was suspected even by MRI. It has thus been shown that biopsies and preoperative imaging results can be false negative in many patients [4]. The carcinoma in our patient was detected at an early T2 stage with no lymph node or solid organ metastasis. This seems to be unusual, as in the report of Iesalnieks *et al.* [4], about 95% of patients had a T3 or even a T4 stage at the time of diagnosis, 50% of whom had documented inguinal lymph node involvement and about 10% presented with distant organ metastasis. Prognosis of CD patients with fistula-associated anal adenocarcinoma is poor. In about 55% of all patients, tumour recurrence occurs after abdominoperineal resection (APR), following a mean duration of 16.6 (2–55) months. Tumour recurrence was observed in 14 of 15 lymph node-positive patients; a positive nodal status is found to be significantly associated with poor outcomes after APR on multivariate analysis [4]. The overall survival rate after APR is 88% at 1 year, 54% at 2 years and 26% at 5 years. Our patient remains tumour-free after about 24 months following APR.

We have previously described that an EMT plays a key role in the pathogenesis of CD-associated fistulae and that EMT-associated molecules, namely, Snail1, Slug, β-catenin and E-cadherin feature a unique expression pattern along the tracts of CD-associated fistulae [9–12]. Interestingly, EMT is also critically involved in the pathogenesis of colorectal and anal carcinoma, and the onset of EMT is a bad prognostic factor [13,14].

To assess a possible involvement of EMT-related events in the pathogenesis of the described CD fistula-associated anal adenocarcinoma, we immunohistochemically stained tissue specimens of the fistula as well as of the carcinoma. These tissue samples were obtained before any therapeutic intervention was performed. The Snail1 transcription factor, a known mediator of EMT, was clearly detectable in the nuclei of myofibroblast-like mesenchymal cells, the so-called transitional cells (TCs), lining the fistula tract in our patient. This is in good accordance with our previous observations [11]. In addition, nuclear, meaning activated, Snail1 protein is detectable in most of the tumour cells (Fig. 2a). However, the Slug transcription factor, which is also associated with

Fig. 2



Immunohistochemical assessment of the Crohn's disease-associated fistula and the mucinous adenocarcinoma. Immunohistochemical analysis revealing staining for (a) Snail1, (b) Slug, (c) β-catenin and (d) E-cadherin in the perianal fistula as well as in the fistula-associated mucinous anal adenocarcinoma. White arrows indicate transitional cells, black arrows indicate cylindrical intestinal epithelial cells and red arrows point to tumour cells.

the onset of EMT, was clearly detected in TCs and in cells below the fistula surface. This is in contrast to our previous observations, as to date we had detected Slug expression only in cells below the surface epithelium but not in TCs [11]. In the carcinoma cells, Slug was strongly detectable (Fig. 2b). Strong staining for β-catenin was detected in IECs along the fistula tract, mainly at the lateral cell membrane. According to our previous observations [10], β-catenin staining was clearly less intense in TCs. With regard to the carcinoma, membrane staining of β-catenin was visible in IECs; however, a large number of tumour cells also revealed nuclear staining of the transcription factor (Fig. 2c) [10]. Similarly, the epithelial protein E-cadherin was also strongly detectable at the membrane of cylindrical IECs along fistula tracts but TCs were clearly less stained, which is in good accordance with our previous findings [10]. However, E-cadherin was strongly detectable in IECs in the carcinoma tissue (Fig. 2d).

These observations confirm our previous findings on the expression of EMT-associated proteins along or around CD-associated fistulae [10–12]. Of note, the Slug transcription factor was also expressed in the TC lining the fistula in this patient, whereas we had previously only found Slug staining in cell layers below the fistula surface. This observation might be because of the carcinoma originating from TCs of the fistula, as Slug expression is also associated with tumour progression. These findings in a patient with fistula-associated mucinous adenocarcinoma of the perianal/perineal region seem to support our previous findings on the expression of EMT-associated proteins in CD-associated fistulas.

Conclusion

Although fistula-associated anal carcinoma is a rare occurrence, this case highlights that chronic fistulizing CD should be considered as precancerosis. A high index of suspicion for malignancy is mandatory in the case of a suspicious perianal or distal rectal mass lesion, even in the absence of malignant cells on histological analysis. Moreover, this case once more envisions that sufficient treatment options for refractory fistulizing CD are still a largely unmet need for a substantial fraction of CD patients.

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Conflicts of interest

There are no conflicts of interest.

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